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Microhabitats, Thermal Heterogeneity, and Patterns of Physiological Stress in the Rocky Intertidal Zone

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Abstract. Thermal stress has been considered to be among the most important determinants of organismal distribution in the rocky intertidal zone. Yet our understanding of how body temperatures experienced under field conditions vary in space and time, and of how these temperatures translate into physiological performance, is still rudimentary. We continuously monitored temperatures at a site in central California for a period of two years, using loggers designed to mimic the thermal characteristics of mussels, *Mytilus californianus*. Model mussel temperatures were recorded on both a horizontal and a vertical, north-facing microsite, and in an adjacent tidepool. We periodically measured levels of heat shock proteins (Hsp70), a measure of thermal stress, from mussels at each microsite. Mussel temperatures were consistently higher on the horizontal surface than on the vertical surface, and differences in body temperature between these sites were reflected in the amount of Hsp70. Seasonal peaks in extreme high temperatures ("acute" high temperatures) did not always coincide with peaks in average daily maxima ("chronic" high temperatures), suggesting that the time history of body temperature may be an important factor in determining levels of thermal stress. Temporal patterns in body temperature during low tide were decoupled from patterns in water temperature, suggesting that water temperature is an ineffective metric of thermal stress for intertidal organisms. This study demonstrates that spatial and temporal variability in thermal stress can be highly complex, and "snapshot" sampling of temperature and biochemical indices may not always be a reliable method for defining thermal stress at a site.

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Introduction

Temperature is one of the most important abiotic determinants of organismal distribution and physiological performance in the rocky intertidal zone (Orton, 1929a, b; Doty, 1946; Hutchins, 1947; Carefoot, 1977; Bertness, 1981; Wethey, 1983, 1984; Menge and Olson, 1990; Williams and Morritt, 1995). Animals and algae in this environment are exposed to rapidly fluctuating and often extreme temperatures, and recent studies have shown that exposure to high temperatures can have significant physiological consequences to these organisms (Hofmann and Somero, 1995, 1996a, b; Stillman and Somero, 1996; Roberts *et al.*, 1997; Chapple *et al.*, 1998; Tomanek and Somero, 1999; Buckley *et al.*, 2001; Dahlhoff *et al.*, 2001; Snyder *et al.*, 2001). Several studies have further indicated that thermal stress can have significant ecological consequences, and that exposure to stressful conditions varies both in space and in time in the rocky intertidal zone. For example, Wethey (1983, 1984) demonstrated that the competitive dominance of one species of barnacle over another varied with substratum angle, presumably as an indirect effect of thermal or desiccation stresses on the relative physiological performance of each species. Menconi *et al.* (1999) found that community structure at an intertidal site in the Mediterranean varied as much as a function of substratum angle as it did as a function of tidal height. Dahlhoff *et al.* (2001) showed that temporal variability in physiological stress had significant effects on the foraging ability of an intertidal gastropod. However, despite a robust and growing body of literature on the physiological ecology of intertidal organisms, we are just beginning to understand on a mechanistic basis how body temperature variation influences physiological performance and, ultimately, how physiological performance contributes to the ecological interactions of intertidal organisms.

Our understanding of temperature effects on intertidal organisms is limited by at least three gaps in our knowledge of the ecological physiology of the rocky intertidal zone. First, although the ecological community is gaining appreciation and insight into the significance of organismal body temperatures under natural field conditions (*e.g.*, Elvin and Gonor, 1979; Wethey, 1983, 1984; Bell, 1995; Williams and Morritt, 1995; Helmuth, 1998, 1999; Dahlhoff *et al.*, 2001), more attention needs to be paid to the complexity of determining spatial and temporal patterns of body temperatures in the intertidal. While it is submerged, an ectothermic invertebrate is likely to have a temperature fairly similar to that of the surrounding water. In contrast, during aerial exposure, climatic factors such as air temperature, wind speed, solar radiation, and relative humidity interact to drive the flux of heat into and out of an organism's body (Johnson, 1975; Bell, 1995; Helmuth, 1998, 1999). As a result, temperature extremes during low tide can far exceed those experienced during submersion, and an organism's body temperature can be substantially different from the temperature of the surrounding air (Helmuth, 1998). Furthermore, heat fluxes are to some extent determined by the size and morphology of the organism. As a result, organisms exposed to identical climatic conditions can experience different body temperatures (*e.g.*, Porter and Gates, 1969; Porter *et al.*, 1973; Helmuth, 1998), and an animal's "thermal regime" is determined in part by its own morphology.

Second, even though accurate determinations of body temperature can be made, the physiologically significant aspect of the "thermal signal" of environmental temperature (*e.g.*, maximum, minimum, average, time history) is unknown. Investigations of the plasticity of physiological processes, such as the environmental induction of heat shock proteins (*e.g.*, Buckley *et al.*, 2001) and the relationship between oxygen consumption and temperature (Widdows, 1976), have documented that invertebrates are responsive to a changing thermal environment in a regulatory manner and therefore must sense environmental temperature. Additional studies have coupled relatively short-term measurements of body temperature to physiological indicators of thermal stress (*e.g.*, Hofmann and Somero, 1995; Tomanek and Somero, 1999; Dahlhoff *et al.*, 2001; Snyder *et al.*, 2001). However, we still do not understand what aspect of environmentally driven body temperature variation is physiologically significant in these ectothermic organisms.

Finally, only recently have advances in technology allowed for measurement of body temperatures as a function of microhabitat over long time scales. Deploying instrumentation in the rocky intertidal zone is notoriously difficult due to damage from waves, and only recently have commercially available instruments become sufficiently small and robust to be deployed for long periods of time. Furthermore, because of the influence of a temperature logger's (or organism's) size, mass, and morphology on the temperature that it records, temperature measurements relevant to intertidal organisms are scarce, and those that exist are not necessarily accurate proxies for the body temperatures of all organisms at that site. Thus, there are relatively few data sets that provide information as to how microscale features of intertidal substrata influence organismal body temperature (except see Wethey, 1983, 1984; Williams and Morritt, 1995; Helmuth and Denny, 1999).

As a first step in addressing these complex issues, we have integrated the fine-scale measurement of organismal body temperature with the analysis of a bioindicator of physiological stress. Specifically, in the current study, we present temperature data recorded by loggers designed to mimic the body temperatures of a competitively dominant mussel, *Mytilus californianus*, and collected over a period of 2 years at a site in central California (Monterey Bay). We couple these data with periodic measurements of isoforms of the 70-kDa heat shock protein (Hsp) gene family, a molecular chaperone that has been used routinely as a bioindicator of stress (see Feder and Hofmann, 1999, for a review), and explore the inherent difficulty in linking patterns in thermal signals in the field to physiological indicators of stress. We further examine the effects of substratum angle on body temperature and levels of thermal stress to address the question of how body temperature and thermal stress vary over small spatial scales in the intertidal.

Our results demonstrate that while biochemical indicators of stress are potentially a very powerful tool for examining the role of environmental variation in driving organismal physiology, we still do not yet have a complete understanding of what aspects of the thermal environment drive the transcriptional activation of stress protein genes. Similarly, high spatial and temporal variability in patterns of body temperatures necessitate caution when extrapolating from short-term measurements of temperature or Hsp production in the intertidal. Namely, while the use of biochemical indicators of stress and concomitant measurements of temperature can potentially serve as an effective link between the ecology and physiology of intertidal organisms, such studies require detailed measurements of body temperature, and an awareness of the potential role of thermal history in driving physiological stress in the rocky intertidal zone.

Materials and Methods

Temperature measurements and logger design

Mussel temperatures were recorded using temperature loggers deployed on the shores adjacent to the Hopkins Marine Station in Pacific Grove, California (37′18.0″ N, 54′ 15.5″ W), from October 1998 to October 2000. Loggers were deployed in the centers of small mussel beds at two microsites in the mid- to high intertidal zone (mean lower low water + 1.7 m): a horizontal, upward-facing microsite

and a vertical, north-facing site. Sites were located within 20 cm of one another, in an area judged to be moderately wave-exposed. A third logger was deployed at the bottom of a nearby tidepool (about 1.5 m × 1 m × 15 cm deep) from July 1999 to June 2000.

Because the same morphological factors that determine heat flux to intertidal plants and animals and drive differences in their body temperatures can also affect heat flux to temperature loggers, we used loggers imbedded in physical models of mussels to collect temperature data. Thus, for example, larger, more massive loggers have a larger thermal inertia than do smaller loggers, and they may not always record peaks in body temperature experienced by animals with a faster thermal response time (Helmuth, 1998). Matching the thermal characteristics of a temperature logger to those of the organism in question is therefore critical, and a single type of logger is unlikely to be an effective proxy for all organisms at an intertidal site. We therefore deployed temperature loggers of a size (60–75 mm) and shape comparable to those of real mussels, and we matched their thermal response characteristics (mass × specific heat) to living animals. From October 1998 to May 2000, empty shells of *Mytilus californianus* (~75 mm in length) were filled with silicone sealant and fitted with a thermistor cable. The recording tip of the thermistor was placed in the center of the silicone-filled mussel shell. The thermistor was then connected to an Onset Corporation Stowaway logger encased in a waterproof housing. Mussels were attached to the substratum in the middle of small beds, in approximate growth position, using marine epoxy (Z-spar). Because of the high rate of damage to thermistor cables, thermistor loggers were replaced in May 2000 with a similar logger designed entirely of epoxy plastic, where the logger (an Onset Corporation Tidbit logger) was encased inside of the fake mussel. Again, the product of mass × specific heat of the plastic logger in the fake mussel was similar to that of a living mussel. Both loggers recorded temperatures to an accuracy and resolution of 0.3 °C, and recorded average temperatures at intervals of 5 to 10 min (preliminary studies indicated that changes in body temperature were slow enough that this sampling interval would capture all peaks). Because logger design was thought to have a significant effect on the temperature recorded only while the logger was exposed to air and not while completely submerged, unmodified Onset Corp. Tidbit loggers were used to record tidepool temperatures.

On 25 days from October 1998 to May 1999, the external logger temperature was compared to the temperatures of living mussels. An infrared thermocouple (Omega Corp.) was used to record the external temperature of the logger, and of 5–10 mussels in the surrounding bed. Results of the 82 comparisons indicated that loggers recorded temperatures that were, on average, within 0.75 °C of those of living mussels, and were usually within 1 standard deviation of the average of the living mussels (correlation analysis indicated a 1:1 curve fit with an R^2 value of 0.94). Temperature data were collected on days in which logger and mussel temperatures ranged from ~11 °C to 27 °C. The loggers were thus thought to serve as a reliable proxy for body temperature, although comparisons were not made for the uppermost range of temperatures observed throughout the year (30–34 °C). Furthermore, because loggers were sealed, they potentially ignored any effects of evaporative cooling due to mussel gaping. However, Bayne *et al.* (1976) showed that aerial respiration by *M. californianus* is generally only effective when relative humidity approaches 100%, when evaporative cooling cannot occur (Helmuth, 1998, 1999). Preliminary experiments (T. Fitzhenry and Helmuth, unpubl. data) also suggest that this species does not gape as a means of evaporatively cooling; nonetheless, this potential complication requires further investigation.

To compare the effects of logger design on temperature recorded, an unmodified Tidbit logger was deployed in the horizontal mussel bed from July 1999 to October 2000. Average and maximum daily temperatures recorded by the unmodified logger were then compared to those recorded by the adjacent physical model.

Temperature analyses

Because of the large number of data points collected by the loggers, temperatures were summarized for each microsite on a monthly basis. Monthly maxima were divided into two categories, each broadly representing a different potential source of thermal stress. "Acute" exposure to high temperature was defined as the absolute maximum temperature experienced by a logger at each site, on a monthly basis (Fig. 1). In contrast, as a measure of "chronic" or repeated exposure to high temperature, the average daily maximum was calculated (Fig. 1). Similarly, the monthly extreme minimum was recorded and average daily minimum was calculated. Other metrics included the daily average temperature (including both aerial and submerged temperatures) and the temperature at high tide (a measure of water temperature). Except for monthly maxima and minima, in which a single point was used for each month, standard deviations of daily average, average daily maximum, average daily minimum, and temperature at high tide were recorded as a metric of variability between days within a month.

Western blot analysis of Hsp70 isoforms

Five specimens of *Mytilus californianus* (length ~50 mm) were collected at each microsite on four dates: 6 July 1999, 24 September 1999, 21 January 2000, and 8 May 2000. Mussels were immediately dissected, and samples of gill tissue were stored at −80 °C until they could be ana-

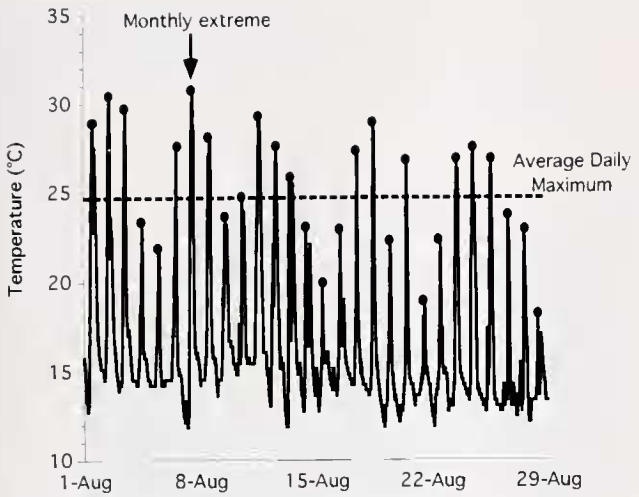

Figure 1. Example of fluctuations in temperature experienced over one month (August 1999) at the horizontal microsite. Daily maxima were calculated from temperature data collected every 5 to 10 min. The highest daily maximum was recorded as the monthly extreme ("acute") high temperature at each site. The average of the daily maxima was calculated as a measure of "chronic" high temperature exposure. Similarly, average daily minima and monthly minima were calculated.

lyzed. Western blotting was employed to determine the levels of both the constitutive and inducible isoforms of Hsp70 in the samples. Hsp70 western blots were performed as described by Hofmann and Somero (1995) except that wet electrophoretic transfer at 30 V for 15 h was used during the western protocol (transfer buffer = 20 m*M* Tris, 192 m*M* glycine, 20% methanol). Equal amounts of protein (10 μg total protein) were separated on 7.5% polyacrylamide gels. A sample of purified Hsc70 (10 ng of bovine Hsc70; Stressgen) was included on each gel as a positive control, and as an internal standard to allow comparison of multiple western blots. Immunodetection was performed using an anti-Hsp70 rat monoclonal antibody that cross-reacts with the cognate and inducible forms of Hsp70 (Affinity Bioreagents; MA3-001). Western blots were developed using an enhanced chemiluminescence protocol according to the manufacturer's instructions (ECL Western Blot Reagent; Amersham) and visualized on a Fluor-S MultiImager (BioRad). Band intensity from each western blot was quantified using Quantity One software. Protein determinations of the gill extracts were performed using a modified Bradford protein assay (Pierce Coomassie Plus).

Levels of Hsp as a function of microsite and of collection date were compared using a two-way analysis of variance. *Post-hoc* comparisons of the effect of season within site, and the effect of site within season, were conducted using a series of one-way ANOVAs with Fisher's PLSD test. Levels of the two isoforms of Hsp70 (Hsp72 and Hsc75, see below) were analyzed separately.

Results

Temperature analysis

Although the study area was superficially judged to be only moderately wave-exposed, wave forces at the sites were often severe (Helmuth and Denny, 1999) and frequently resulted in the loss of or damage to loggers. Gaps in the data sets are therefore present, particularly during winter months when wave forces were greatest. Summary statistics for months in which fewer than 3 weeks of data were collected are thus not reported.

From 11 December 1999 to 6 May 2000, the only loggers recovered at the horizontal site were the unmodified Tidbit loggers. A correlation analysis from days on which both the unmodified and modified loggers were present at the horizontal site (n = 329 days) indicated that temperatures recorded by the unmodified logger could be used to predict those recorded by the physical models (R^2 = 0.96). An offset value (+0.46 °C) calculated from the correlation analysis was used to predict maximum daily temperature for the missing 149 days, and an offset of +0.15° was used to predict average daily temperature. No correction was required for predicting minimum temperatures. On any given day, however, maximum temperatures recorded by the two loggers differed by as much as 4.7 °C, with an average difference of 1.3 °C. The correlation between the unmodified logger and the logger on the north-facing substratum was too poor to be useful for days in which the logger at that site was missing.

The highest annual temperatures at the horizontal microsite (Fig. 2a) were recorded in May 1999 (33.8 °C on 23 May) and August 2000 (33.8 °C on 10 August). The highest levels of "chronic" high temperature exposure (average daily maxima) at this microsite were recorded in August 1999 (24.4 °C), and in June 2000 (24.2 °C; Fig. 2a). Thus, the levels of these two metrics of temperature exposure were out of phase with one another, most obviously in 1999 (Fig. 2a). In contrast, on the north-facing site, both the highest average daily maximum and the yearly extreme high temperature (29.1 °C on 10 August) occurred in August 1999 (Fig. 2b); insufficient data were collected to assess the timing of the extremes at the north-facing microsite in 2000. Minimum temperatures were comparable between the north-facing and horizontal microsites, and tended to occur during aerial exposure after sunset. Notably, two freeze (or near freeze) events were recorded on the early evenings of 22 and 23 December 1998, with loggers on the north-facing sites recording temperatures of about −0.6 to −0.9 °C. A large disturbance in the mussel bed was recorded a few weeks later; whether it was precipitated by the freeze is unknown (Helmuth and M. W. Denny, Stanford University, unpubl. data).

Temperatures were consistently higher at the horizontal site than at the north-facing site (Figs. 3 and 4). On average,

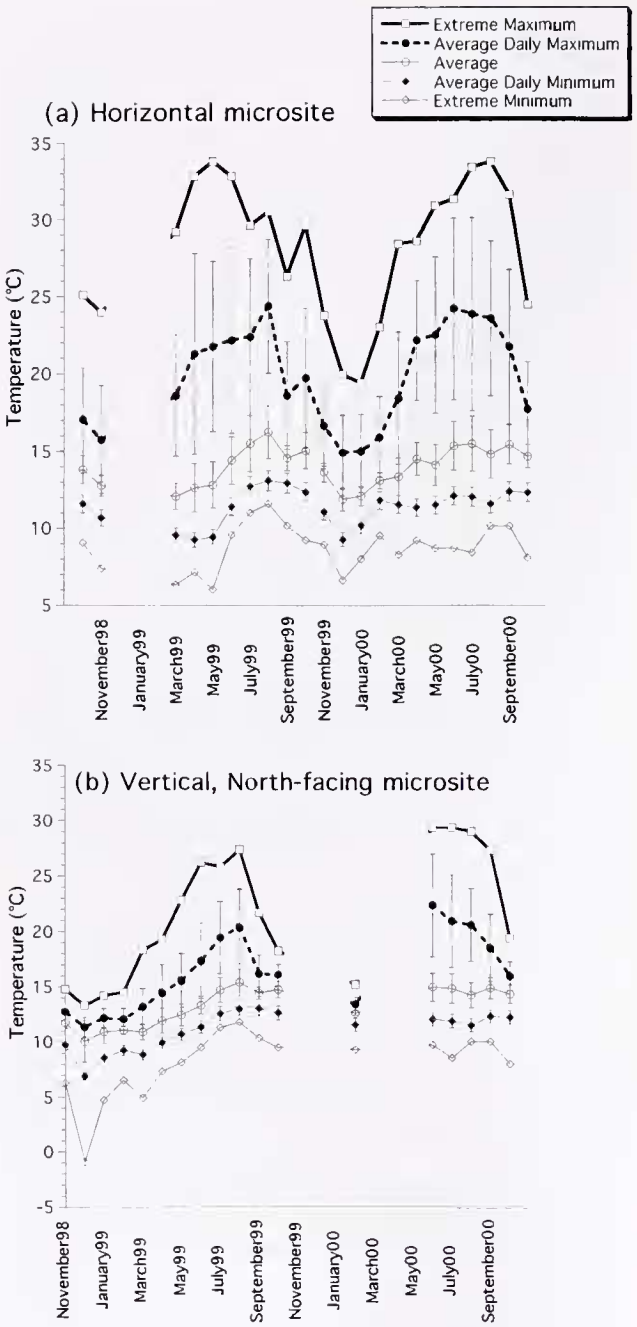

Figure 2. Temperature statistics recorded at the (a) horizontal microsite and (b) vertical, north-facing microsite from November 1998 to October 2000. Temperature data from January to May 2000 at the horizontal microsite were extrapolated from an unmodified logger placed in the bed. Yearly maxima at the horizontal site occurred in May 1999 and August 2000. In contrast, peaks in the average daily maximum ("chronic" temperature exposure) at this site occurred in August 1999 and June 2000. Standard deviations indicate the amount of variability within each month, except for monthly extremes and minimums, for which a single point was recorded during each month-long interval. At the north-facing site (b) the yearly maximum and the highest average daily maximum occurred in August 1999. Note the incidence of an unusual freeze event in December 1998.

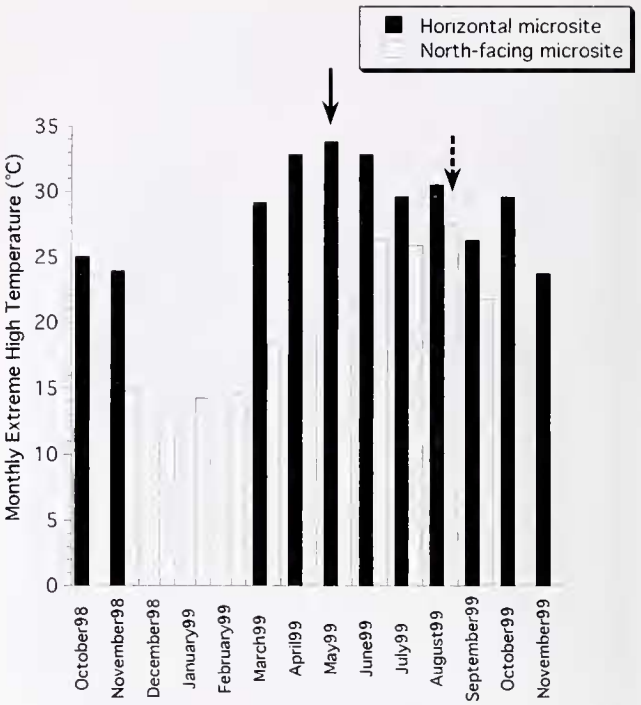

Figure 3. Comparison of monthly high extreme temperatures recorded on horizontal and north-facing microsites. Monthly extrema were always highest on the horizontal substrate, in some months by 10 °C or more. The seasonal timing of temperature maxima varied between sites, occurring in May 1999 on the horizontal site and in August 1999 on the vertical site (indicated by arrows).

extreme maximum monthly temperatures recorded on the horizontal site were 6.75 °C hotter than those on the vertical site; the difference in extreme high monthly temperatures between the horizontal and vertical sites ranged from a high of 13.5 °C in April 1999 to a low of 1.9 °C in June 2000 (Fig. 3). Average daily maxima calculated for each month were also higher on the horizontal site, with an average difference of 3.6 °C, ranging from 1.7 °C in October 2000 to 6.8 °C in April 1999 (Fig. 4).

Temperatures recorded in the tidepool were not as high as those on the exposed horizontal microsite, but in general exceeded those recorded on the aerially exposed vertical face (Fig. 5). Both the yearly extreme high temperature maximum (29.8 °C on 2 August 1999) and the highest average daily maximum (24.5 °C) in the tidepool were recorded in August 1999 (Fig. 5). Water temperatures (recorded by the loggers at high tide) were highest in August through October 1999 and July through September 2000 (~15 °C), and displayed a pattern that was markedly different from any of those recorded during aerial exposure (Fig. 6).

Heat shock protein analysis

To compare the physiological status of mussels from the different microsites, the cellular levels of isoforms of the

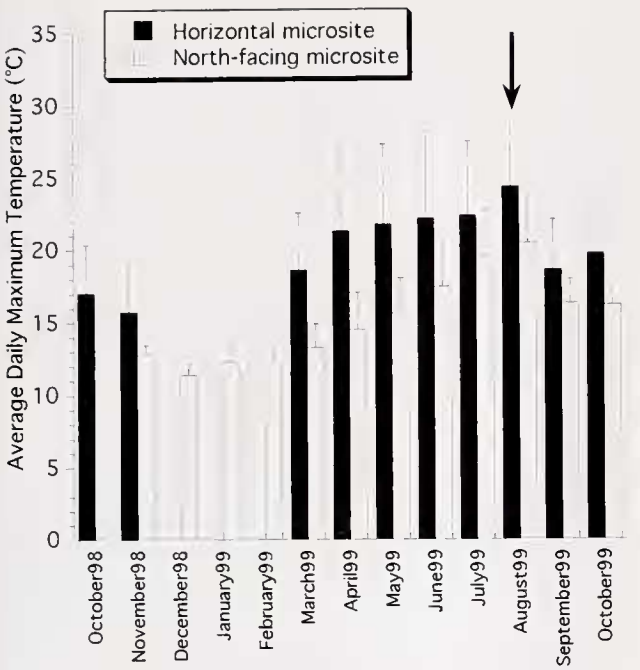

Figure 4. Comparison of average daily maxima recorded at the two aerially exposed sites. Again, levels of "chronic" high temperature exposure were highest on the horizontal substratum. In both cases, highest yearly levels in 1999 occurred in August.

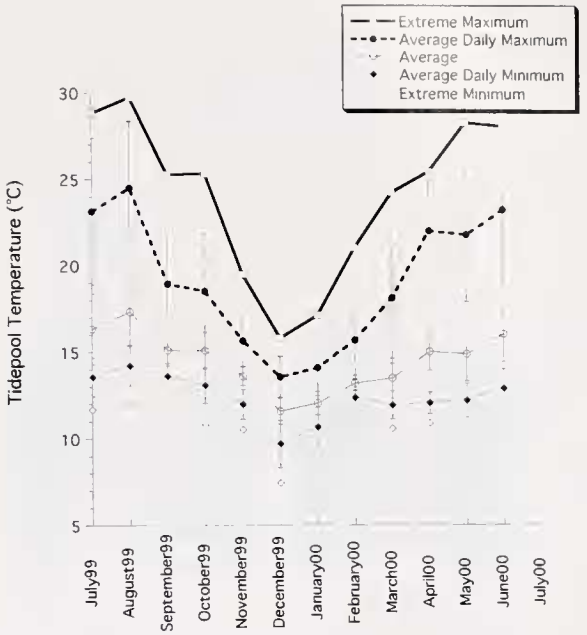

Figure 5. Temperature recorded in a small tidepool. As expected, temperature extremes were buffered relative to the aerially exposed horizontal substratum. However, high temperature extremes were higher than those on the aerially exposed, north-facing site, with yearly extremes reaching nearly 30 °C.

70-kDa Hsp gene family were measured in gill tissue of mussels collected in each season of the year—in July and September 1999, and January and May 2000. Figures 7 and 8 show relative endogenous levels of isoforms of Hsp70 in two groups that separate on SDS-PAGE, a 72-kDa band (Fig. 7) and a 75-kDa band (Fig. 8). Although the precise identity of the separate proteins that compose the two sets is unknown and cannot be determined using one-dimensional electrophoresis, the two isoforms display changes that to some degree correspond to the temperature exposure of *Mytilus*. In previous studies, the 72-kDa band varied significantly with the thermal history of the mussel, with higher levels in summer than in winter; in contrast, the higher molecular mass band varied less as a function of season (Hofmann and Somero, 1995; Roberts *et al.*, 1997). Therefore, we have expressed the data using the two sets of isoforms as separate indicators, where the 75-kDa band (hereafter Hsc75) reflects constitutive levels of Hsp expression and the 72-kDa band (hereafter Hsp72) reflects a stress-inducible subset of the 70-kDa Hsps.

Overall, levels of the 70-kDa molecular chaperones in mussel gill varied significantly as a function of microsite (Table 1; Figs. 7, 8). Regardless of season, levels of Hsp72 were always significantly greater in mussels on the horizontal substratum than in mussels attached to the north-facing surfaces of rocks (ANOVA; $P = 0.0001$; Fig. 7; Table 1). However, there was no consistent pattern for Hsc75 (Fig. 8). Compared to north-facing mussels, the horizontal mussels had significantly higher levels of Hsc75 only in January ($P = 0.012$). In July, Hsc75 levels in the two groups were

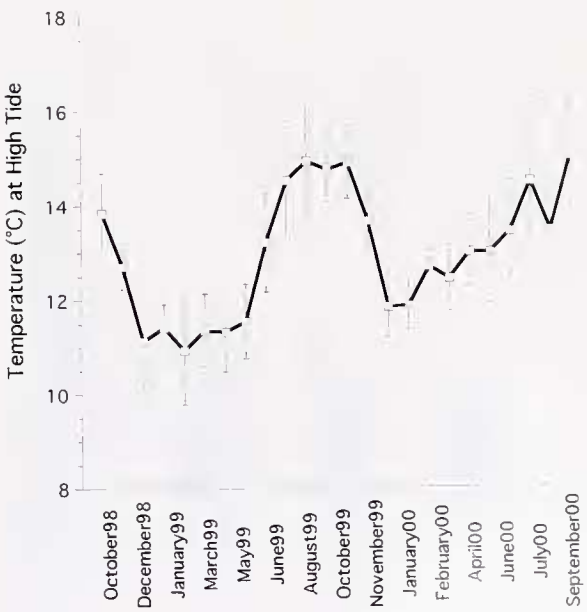

Figure 6. Patterns in water temperature recorded during high tide. The seasonal pattern in water temperature is markedly different in both magnitude and timing from those recorded in any of the microsites during aerial exposure.

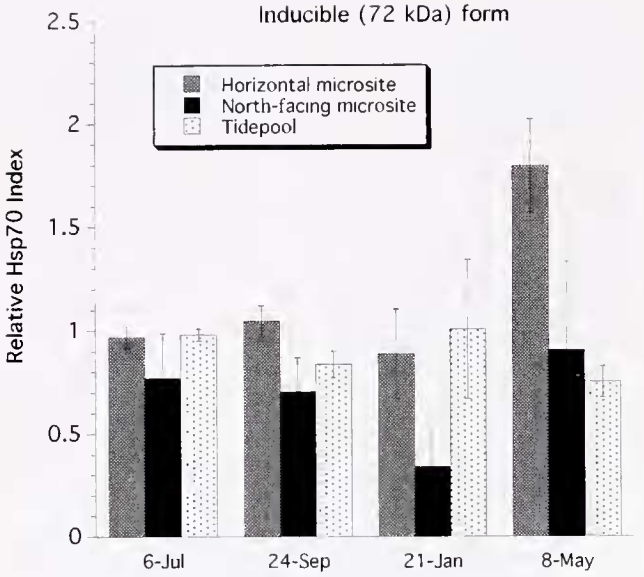

Figure 7. "Inducible" (72-kDa isoform) levels of heat shock protein from mussels collected at each of the three sites. See Table 1 for statistical results, and Table 2 for temperature conditions experienced by mussels prior to each collection. In general, inducible forms were significantly higher in mussels from the horizontal site than in mussels from the north-facing site. Differences between the aerially exposed mussels and mussels from the tidepool were less consistent.

equivalent, and in the other two months the levels were significantly lower in the horizontal mussels than in the north-facing mussels (September, $P = 0.0001$; May, $P = 0.0001$; Fig. 8).

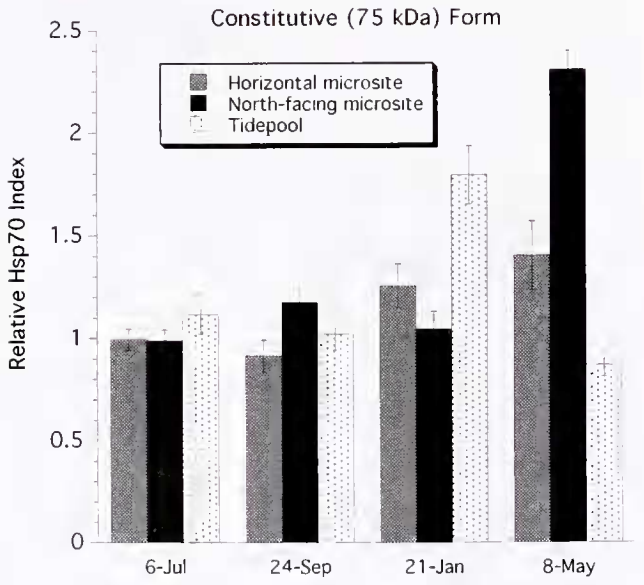

Figure 8. "Constitutive" (75-kDa isoform) levels of stress proteins from mussels at each site. Constitutive forms are thought to be affected by multiple physiological parameters and do not necessarily change with thermal stress. See Table 1 for results of statistical analysis.

Table 1

Results of statistical analyses of the 72-kDa form of Hsp 70

Effect of Site within Collection Date*	
6 July 1999	H = TP > N ($F = 4.12$, $P = 0.0400$)
24 September 1999	H > TP = N ($F = 12.14$, $P = 0.0013$)
21 January 2000	H = TP > N ($F = 9.64$, $P = 0.0030$)
8 May 2000	H > TP = N ($F = 19.85$, $P = 0.0002$)
Effect of Collection Date within Site	
Horizontal	May > July = Sept. = Jan. ($F = 33.0$, $P = 0.0001$)
North-facing	July = Sept. = May > Jan. ($F = 4.0$, $P = 0.0270$)
Tidepool	July = Sept. = Jan. = May ($F = 2.4$, $P = N.S.$)

Overall analysis using a two-factor ANOVA indicated a significant effect of collection date ($F = 10.0$, $P = 0.0001$), Site ($F = 28.2$, $P = 0.0001$) and a significant interaction term ($F = 9.23$, $P = 0.0001$). A series of one-way ANOVAs with Fisher's PLSD *post hoc* tests were used to discern the effects of site within collection date, and of collection date within site.

* H, horizontal; TP, tidepool; N, north-facing.

In a comparison of tidally exposed and constantly submersed individuals, there were no obvious differences or trends in either Hsp72 or Hsc75 levels between the horizontal and north-facing mussels and mussels that were permanently immersed in a tidepool (Figs. 7, 8). Hsp72 levels were greater in horizontal mussels in September and May as compared to tidepool mussels, but these levels were equal in July and January (Fig. 7). Hsp72 levels in tidepool mussels were equivalent to north-facing levels in May and September, but tidepool mussels had significantly greater levels in July and January than did their north-facing counterparts. For Hsc75, levels in mussels from the tidepool were greater than those in horizontal mussels in September, July, and January, and significantly lower than in mussels from horizontal surfaces in May. Levels of Hsc75 from tidepool mussels were significantly greater than in north-facing mussels in July and January, and significantly lower than in north-facing mussels in May and September.

Finally, the three microsites displayed variation in levels of the 70-kDa Hsp bioindicators as a function of time of collection (Figs. 7, 8). For the horizontal mussels, Hsp72 levels were higher in May than in any other month; however, all three other months (July, September, and January) were not significantly different from each other (Fig. 7). In contrast, the mussels from the north-facing substratum had their lowest levels of Hsp72 in January; the difference between January and the other months was statistically significant. Hsp72 levels in gill from north-facing mussels were not significantly different amongst the July, September, and May collections. With respect to Hsc75, horizontal mussels in September and July had equivalent but lower

Table 2

Temperature measurements conducted during the week prior to each mussel collection

	Horizontal	North-facing	Tidepool
30 June–6 July 1999	19.8 (25.1)	20.5 (26.3)	22.0 (26.3)
18–24 September 1999	21.1 (26.3)	17.1 (19.1)	20.7 (25.3)
15–21 January 2000	13.0 (15.3)	N.R.	14.9 (16.1)
2–8 May 2000	20.9 (30.0*)	14.2 (22.0)	19.0 (26.6)

Both the average daily maximum temperature and the extreme temperature (in parentheses) recorded during that week are given (°C).

* The 30 °C temperature recorded on May 8 was for a very brief period of time (<20 min.).

levels than in January and May; north-facing mussels displayed the highest values in May as compared to all other months, which were not significantly different from each other. Interestingly, the tidepool mussels exhibited no seasonal effect on Hsp72 levels (Fig. 7), but they did show some variation in Hsc75 levels (Fig. 8). Specifically, the levels of Hsc75 in May and September were equivalent to each other but significantly lower than in the months of July and January ($P = 0.001$); July and January levels were not significantly different from each other.

A comparison between the maximum temperature exposure in the week prior to collection (Table 2) and the levels of Hsp72 (Fig. 7), shows that inducible Hsp levels generally increased with maximum temperature exposure, but the correlation was not as good as might be expected (Fig. 9). A regression of Hsp72 with maximum temperature indicated a significance level of $P = 0.03$ (Statview; $F = 7.66$) when both north-facing and horizontal mussels were considered (note that the temperature datum for the January north-facing site was assumed to be no higher than that on the horizontal site). Tidepool data (not shown) generally fell along the same trend line, but reduced the significance level to $P = 0.059$ ($F = 4.55$).

Discussion

Intertidal organisms live at the margins of the marine and terrestrial environments and must contend with the changing physical conditions of both regimes. Recently, much attention has been paid to the influence of seawater temperature, and in particular to changes in seawater temperature as a result of climate, on changes in intertidal communities (*e.g.*, Barry *et al.*, 1995; Sagarin *et al.*, 1999). However, few studies have investigated the importance of aerial exposure to intertidal organisms in a changing thermal environment (but see Denny and Paine, 1998). Clearly, extremes in body temperature (both high and low) experienced during exposure to air far exceed those occurring during high tide. Depending on the zonational height of the organism, the duration of exposure to air can be as long as or even longer than the submersion time.

An important question that remains to be answered is, how important to an organism's physiological performance is thermal stress during low tide as opposed to the effects of water temperature during submersion? Previous evidence suggests that some intertidal organisms slow their metabolic rates during aerial exposure, and in some cases resort to anaerobic metabolism (*e.g.*, Bayne *et al.*, 1976). Work by Sanford (1999) has suggested that the rate of predation by the sea star *Pisaster* is driven by water temperature and appears to be unrelated to air temperature. In contrast, measurements of Hsp production show that the temperatures at which Hsps are induced occur almost exclusively during low tide (*e.g.*, Roberts *et al.*, 1997; Tomanek and Somero, 1999), and that the deficit to the protein pool can have a significant effect on the animal's scope for growth (Roberts *et al.*, 1997). Mass mortalities due to thermal stress also have been reported primarily as a result of extremes in temperature experienced during exposure to air (*e.g.*, Glynn, 1968; Suchanek, 1978; Tsuchiya, 1983; Liu and Morton, 1994; Williams and Morritt, 1995). Understanding the relative importance of thermal stress during submersion *versus* during aerial exposure is therefore key if we are to decipher

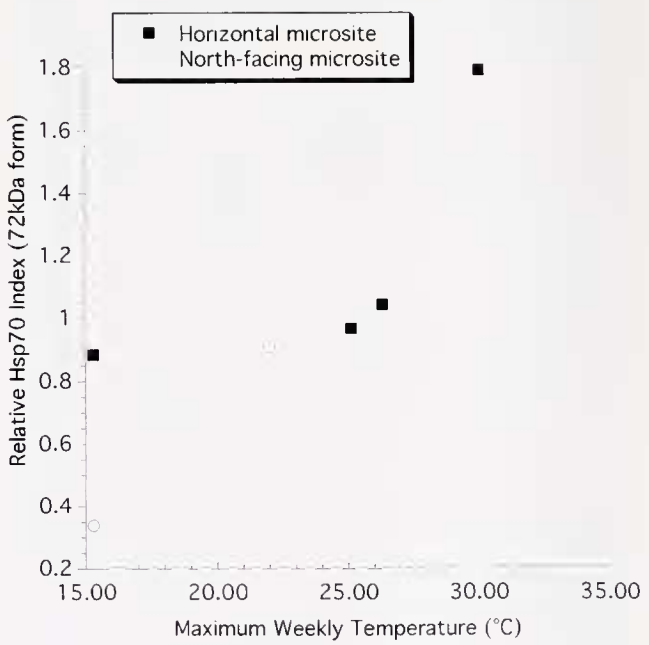

Figure 9. Comparison of Hsp72 ("inducible form") levels *vs.* the maximum temperature recorded in the week prior to collection. As a result of data logger failure, no temperature data were collected on the north-facing site prior to the January collection. For the purposes of this figure, we thus assume that the north-facing site was no hotter (15.3 °C) than the horizontal site where temperatures were recorded. A simple regression reveals a significant relationship between Hsp72 and maximum temperature ($P = 0.03$; $F = 7.66$), although it should be noted that the relationship is significant primarily because of the large spike in Hsp72 production observed in May.

and predict the effects of climate, and of climate change, on intertidal communities.

Our results show that patterns in body temperature experienced during low tide cannot be predicted on the basis of measurements of nearshore water temperature. Similarly, preliminary evidence (Helmuth, unpubl. data) suggests that air temperature is also an ineffective proxy for body temperature. This observation is pertinent because air and water temperatures are frequently the dominant metrics used to estimate patterns in thermal stress in the intertidal zone (*e.g.*, Barry *et al.*, 1995; Menge *et al.*, 1997; Sagarin *et al.*, 1999; Denny and Paine, 1998; Sanford, 1999; Thompson *et al.*, 2000). Furthermore, as our data indicate, high spatial variability due to substratum angle can lead to large differences in body temperatures. Single measurements of temperature, and particularly those based on water or air temperature, cannot be used to define thermal stress at an intertidal site or to compare multi-year trends in community structure as a function of climate change.

Our study also points out gaps in our understanding of what aspect of the thermal environment drives organismal stress and of how organisms respond to temporally varying environmental signals. Widdows (1976), for example, showed that, when acclimated to cyclic temperatures, *Mytilus edulis* decreased its amplitude of response of filtration rate and oxygen consumption to changing temperatures. More relevant to our study, previous research has shown that the threshold induction temperature and the total cellular pools of Hsps in mussels changed as a function of season and thermal acclimation in the laboratory (Roberts *et al.*, 1997; Buckley *et al.*, 2001). Although these studies clearly demonstrate an effect of thermal history on the physiology and regulation of the heat shock response, the mechanism that couples variation in environmental temperature with the physiological response is unknown. Surprisingly, even in the heat shock biology of model cells, there is no consensus about how the thermal signal is transduced from the membrane, through protein kinase cascades to the nucleus (*e.g.*, Lin *et al.*, 1997; Ng and Bogoyevitch, 2000; Han *et al.*, 2001). As ecological physiologists, if we are ever to determine the pathway of signal transduction of temperature in an organism in nature, we must first understand the physiologically important aspect of temperature.

Thus, one of the goals of this study was to bridge the gap between temperature exposure in nature and a predictable molecular response, the heat shock response. Our results highlight the complexity of examining an environmentally induced gene expression event in organisms in a natural population. Although there are some instances in which the Hsp levels "match" the predicted result (Fig. 9), there are others in which the correspondence is poor. For example, as expected, mussels living on horizontal substrata consistently had higher levels of Hsp72, the inducible isoform of the 70-kDa Hsp gene family, than did mussels on north-facing substrata (Fig. 7, Tables 1, 2). In contrast, seasonal differences in Hsp production (Fig. 7) were less easily interpreted and did not always display the pattern observed in other studies of intertidal mussels. For example, levels of Hsp72 in mussels from the horizontal microsite were nearly as high (albeit more variable) in January as in July (Fig. 7), even though recorded body temperatures were considerably higher in July than in January (Table 2). On the other hand, higher temperatures recorded in May (Table 2) were reflected in Hsp72 production during this time period (Figs. 7, 9), and appear to be most closely related to differences in extreme temperature (30 *vs.* 25 °C; Table 2). These patterns, and in particular the patterns observed in Hsp75 production, suggest again that there are numerous factors at work in the control of chaperone levels and that thermal stress may not be the only factor driving variation in Hsp expression. Specifically, other physiological stressors such as hypoxia and desiccation may contribute to temperature's influence on Hsp induction (see Feder and Hofmann, 1999). Furthermore, our study shows that the seasonal timings of potential stressors do not always act in concert, and that the timing of "acute" and "chronic" high temperature exposures varies with substratum angle. The thermal landscape is highly variable, and conclusions drawn from any given study could depend on the sampling regime (*e.g.*, effects of substratum angle). Extreme caution must be exercised when collecting samples over limited spatial and temporal scales as a means of defining thermal stress at a site.

Our data also address the inherent complexity of using Hsps as biomarkers in the environment. In general, the heat shock response is subject to complex regulation in the cell (Kline and Morimoto, 1997; Ali *et al.*, 1998; Morimoto, 1998; Zhong *et al.*, 1998). The nature of Hsp gene activation can change with the length and severity of the thermal stress (see Lindquist, 1986, for a review; see also Yost *et al.*, 1990), and Hsp70 mRNA stability varies as a function of temperature (Petersen and Lindquist, 1988, 1990). In fact, Hsps are thought to control their own expression *via* a negative feedback loop, making the cellular pools and the induction points interrelated (*e.g.*, DiDominico *et al.*, 1982; Craig and Gross, 1991; Shi *et al.*, 1998). Furthermore, once the Hsps are synthesized, they are also subject to decay just like any other protein, and their half-life is influenced by the thermal conditions of the cell. In combination, all the mechanistic and complex regulatory aspects of the heat shock response make for a system that not only is sensitive to temperature but also is directly influenced by temperature, just like any other biomolecular process in a cell. Thus, for example, mussels exposed to lower chronic levels of high temperature may produce inducible forms of Hsp at a lower acute temperature level than will mussels that were acclimated to high average daily maxima. Thus, a hot day that is preceded by a week of relatively mild days may elicit a very different physiological response than an extreme tempera-

ture exposure that follows several days of gradually increasing daily maxima. In summary, the effects of both extreme temperature events (acute temperature exposure) and of the thermal history (*e.g.*, chronic temperature exposure) are likely to be important, but we do not yet sufficiently understand the molecular consequences of temperature variation or how variation in signal transduction and in gene expression would alter the pools of Hsps.

In some ways our study raises more questions than it answers. Defining "thermal stress" at any given site is likely to be complex. Substratum angle can have an enormous effect on the magnitude, timing, and thermal history of temperature. Because ectothermic organisms influence their body temperatures at least partially through their size and morphology, two organisms at one site might experience different patterns in the thermal signal, particularly if they are mobile (*e.g.*, Orton, 1929a). Thermal stress may therefore be organism-specific, rather than site-specific (Menge and Olson, 1990). Finally, care must be taken to account for the thermal conditions occurring during the collection period. Thermal stress experienced during low tide results from the interaction between terrestrial climate and the timing of low tides as set by the tidal series (Orton, 1929a; Helmuth, 1999). For example, sites separated by tens of kilometers have been predicted to experience temperature maxima that differ by several degrees due to the timing of low tide during the hottest times of the year; organisms at sites where low tide occurs at noon may experience much higher temperatures than those at sites where low tide occurs in the morning (Helmuth, 1998, 1999). Inter-annual and decadal-scale variations in tidal exposure have also been predicted to occur (Denny and Paine, 1998). The coupling of biochemical indicators of stress with detailed measurements of temperature may be effective in predicting the role of climate in driving the ecology of rocky intertidal communities, and in predicting the effects of climate change on these ecosystems. However, for ecologists, the temptation to base large-scale comparisons of the role of thermal stress on limited measurements of stress proteins must be balanced by a knowledge of the role of the organism's "cellular thermostat" in driving its physiological response to temperature change. Conversely, physiologists must have a better grasp of how temperatures change in nature if we are to extrapolate from controlled laboratory experiments to conditions in the field. Thus, while there is no simple mechanism for linking patterns in temperature to patterns in physiological stress, the merger of these levels of approach promises to be fruitful for understanding the effects of climate on the rocky intertidal zone.

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